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THE EFFECT OF MUSTARD GAS (DICHLORETHYLSULPHID) ON ANTIBODY FORMATION

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Mustard gas was discovered by Victor Meyer [1] but its poisonous effects attracted little attention until 1917 when it was used by the Germans at Ypres, and since then much has been written about it from various points of view.[2]

Meyer noted that this substance had a specific toxic action on the skin, conjunctiva and respiratory tract, and he concluded that the most severe action develops only after its entrance into the blood. The toxicity, he found, depends on the chlorine content, the monochlorethylsulphid being less toxic than the dichlorethylsulphid. Man seems to be more susceptible than animals and to reveal a greater individual difference, so far as the skin is concerned.[3] The extreme toxicity of mustard gas is shown by the fact that the human eye may react after an exposure of less than one hour to one part in 10,000,000.[4] That the hematopoietic system is affected is indicated by the leukopenia quickly produced by the gas. Krumbhaar and Krumbhaar,[5] from observations on 108 victims whose blood was studied and in 75 necropsies, in 55 of which the bone marrow was examined, conclude:

"1. Yellow cross or mustard gas exerts on the bone marrow a direct toxic action, which, by depleting the leukocytes of the circulation, has an important bearing on the inability to resist secondary infection that is found in that form of gas poisoning. 2. This toxic action on the bone marrow is shown not only by small areas of necrosis, but by an inhibition of the regenerative process (chiefly of the leukogenetic series). 3. Not only is the amount of regenerative hyperplasia inadequate to the severity of the process (as compared with the marrow hyperplasia of various acute infections), but also the quality is inferior, that is, the great majority of the homopoietic cells present are of immature types."

They found also that the period of highest mortality in man coincides with the most severe leukopenia, and regard the action on the bone marrow as similar to that of benzene.

More recently Pappenheimer and Vance,[6] in a study of the effects of intravenous injection of dichlorethylsulphid in rabbits, found the lethal dose thus given to be 0.005 to 0.01 gm. per kilo. Animals thus treated and dying within

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[1] Ber. d. deutsch. chem. Gesellsch., 1886, 19, p. 3259, and 1887, 20, p. 1729.

[2] For a complete bibliography to 1919 the excellent monograph "Mustard Gas Poisoning," by A. S. Warthin and C. V. Weller, 1919, may be consulted.

[3] Eyster, J. A. E., and Maver, Mary E.: Jour. Pharm. and Exper. Therap., 1920, 15, p. 95.

[4] Reed, C. I.: Ibid., 1920, 15, p. 77.

[5] Jour. Med. Res., 1919, 40, p. 497.

[6] J. Exper. Med., 1920, 31, p. 72.

24 hours showed extensive hemorrhages and edema of the lungs, and about one-third presented severe lesions of the intestinal tract. Dichlorethylsulphid injected intravenously was specifically poisonous for the hematopoietic tissues producing severe lesions in the marrow with a marked diminution of circulating leukocytes. The granular cells of the marrow seemed to be more sensitive than the lymphoid cells and erythrocytes. They found that this effect of mustard gas on the blood and hematopoietic tissues was not due to the admixture of nitrobenzene or chlorobenzene. They used a freshly prepared suspension in 30% alcohol in distilled water made from a 10% solution in absolute alcohol, and to avoid hydrolysis of the mustard gas, which occurs rapidly in aqueous suspensions, the injection was made immediately after dilution. Warthin and Weller confirm these observations.

Lynch, Smith and Marshall[7] found a dose of 6 mg. per kilo injected intravenously in aqueous solution to be fatal for dogs. Their aqueous solutions proved markedly unstable, 15% hydrolyzing in 10 minutes at 10 C., while at 37.5 C., over 97% was decomposed in the same time. They found that mustard gas is excreted in the urine, in part at least, as dihydroxyethylsulphid, a comparatively nontoxic body, and advance the theory of action that the dichlorethylsulphid penetrates the cells, and in the aqueous phase of the cell hydrolyzes to hydrochloric acid, which is responsible for the damage.

In the early studies on the circulating leukocytes after mustard gas poisoning, the leukopenia was apparently not noted, but Krumbhaar[8] called attention to this in man, especially to be noted in severe or fatal cases. At first there is an increase in both the red and white count (in individual cases as high as 36,000 and averaging in his series about 12,000) not to be attributed to the increased blood concentration. The Arneth scale is distinctly shifted to the left and then shifts to the right indicating an exhaustion of the leukocyte forming centers. If death does not interrupt the downward course, an extreme degree of leukopenia (at the expense of the polymorphonuclears) may be reached. With recovery, the leukocytes gradually rise to normal or slightly above. An initial rise in erythrocytes is later replaced by a moderate anemia. In the later leukopenic stage, the blood platelets become sparser. Pappenheimer and Vance[6] found unexplained variations in the percentage leukocyte counts of gassed rabbits. In rabbits surviving more than 24 hours after a single injection of 0.005 to 0.01 gm. of mustard gas per kilo there was a pronounced fall in the number of circulating leukocytes. Even in the extreme leukopenia, the rare leukocytes were normal morphologically. There was an absolute and percentage increase in the polymorphonuclears, which fell rapidly with the onset of the leukopenia. In the terminal stages the polymorphonuclears disappeared from the peripheral blood. Sometimes the initial fall in number and percentage of polymorphonuclears is followed by a relative and percentage increase. This secondary rise is associated with the appearance of "unripe" forms in considerable numbers. The leukopenia is accompanied by a relative lymphocytosis. The absolute number of lymphocytes is diminished in the later stages and lags behind that of the granular cells in the cases in which regeneration occurs. There is a percentage increase in the large mononuclear cells, but their absolute number is unchanged or diminished. There is a moderate anisocytosis of the erythrocytes in the terminal stages and during recovery numerous normoblasts appear.

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[7] Jour. Pharm. and Exper. Therap., 1918-19, 12, p. 265.

[8] Jour. Am. Med. Assn., 1919, 72, p. 39.

So far as we are aware, no observations have been made on the effects of mustard gas on antibody formation. In view of its action on the marrow, which resembles to some extent the actions of benzene, of the roentgen ray and of thorium X, all of which have been found to interfere with the production of antibodies, it seemed to us that it might be of interest to study mustard gas from this point of view also. Accordingly, the following experiments have been made on rabbits and dogs, the technic for estimating the antibody content of the serum being the same as in the previous experiments along the same general line.[9] In the tables the figures give the highest active dilutions of serum with respect to lysin and agglutinin and the highest dilution of antigenic serum giving the precipitate reaction with the serum of animals experimented with.

The mustard gas was obtained from Dr. J. A. E. Eyster of the University of Wisconsin. On account of the rapid hydrolysis in watery solution, we used 0.1% solutions in 50% glycerol, which remain stable at least for some hours. Preliminary tests showed that 0.005 c c of mustard gas in 50% of glycerol per kilo is lethal for rabbits when injected intravenously and that 0.003 c c per kilo is lethal for dogs.

EXPER. 1.—(Belgian hares weighing from 3½ to 4½ kilograms.) Rabbits were given various doses of mustard gas intravenously a few days before and after, as well as the same time as they received 30 c c of sheep blood intraperitoneally. The titer of the serum in antisheep lysin and precipitin was determined in the usual way, at first daily and later at longer intervals. While this experiment was in progress, an epidemic of snuffles broke out among the rabbits in the laboratory on account of which the results may have been affected to a certain extent, but in view of the results of control observations and of later experiments, the effect of the epidemic evidently was of minor importance at the most. In the animals that received mustard gas (0.005, 0.001, 0.0001 c c) 4 days before the antigen, in all cases less lysin and precipitin were produced than in the controls. The lysin curve in the animals that received mustard gas ran about the usual course, but the latent period of the precipitin curve was much longer than usual; in one case no precipitin appeared until the 13th day and in two other animals precipitin was not demonstrable until the 9th day, while in the controls it was demonstrable on the 6th or 7th day. The effects outlined were obtained in about the same degree regardless of the amount of mustard gas, but the reduction in the number of leukocytes in the blood was greatest by far in the rabbits receiving the largest amount of mustard gas (0.005). Practically the same effects were obtained in the animals that received the mustard gas at the same time as the antigen. Here also a prolongation of the latent period in precipitin production was noticeable. Given 4 days after the antigen, the mustard gas still seemed to have some restraining effect on antibody production. The results are illustrated in table 1.

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[9] Hektoen: J. Infect. Dis., 1916, 19, p. 69; 1915, 17, p. 415; 1918, 22, p. 28. Hektoen and Corper: ibid., 1920, 26, p. 330.

EXPER. 2.—In this experiment, also in experiments on rabbits (domestic), only one dose, namely, 0.001 c c of mustard gas was given and the amount of antigen was 20 c c. No epidemic influenced the results in any way. The injection of 0.001 c c of mustard gas 5 days before the antigen had a more marked effect on the production of lysin and precipitin, the lysin curve being especially irregular, than in the animals that received the mustard gas with the antigen or 5 days later than the antigen (table 2).

TABLE 1

EFFECTS OF MUSTARD GAS ON ANTIBODY FORMATION IN RABBITS

Days After Injection of Sheep Blood (30 c c)	Mustard Gas, 0.005 c c Given 4 Days Before Injection of Sheep Blood		Mustard Gas, 0.005 c c Given at Same Time as Injection of Sheep Blood		Mustard Gas, 0.001 c c Given 4 Days After Injection of Sheep Blood		Control	
	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin
1	192	0	0	0	96	0		
2	192	0	96	0	192	0	384	0
3	1,536	0	48	0	192	0		
4	1,536	0	192	0	384	0	1,536	0
5	3,072	0	768	0	1,536	0		
6	3,072	0	768	0	1,536	0		
7	3,072	0	768	0	1,536	400	3 072	3,200
9	1,536	0	768	400	6,144	800	6,144	3,200
11	3,072	0	384	200	6,144	3,200	12,288	3,200
13	3,072	1,600	48	200	6,144	1,600	6,144	6,400
15	3,072	1,600	24	0	6,144	3,200	6,144	6,400
18	3,072	1,600	96	0	6,144	1,600	6,144	6,400
20	3,072	6,400	192	0	6,144	1,600	6,144	6,400
22	3,072	6,400	192	0	6,144	1,600	6,144	6,400
25	384	6,400	192	0	.....	.....	6,144	6,400
27	384	1,600	96	0				

TABLE 2

EFFECT OF 0.001 C C OF MUSTARD GAS ON ANTIBODY FORMATION IN RABBITS

Days After Injection of Sheep Blood	Mustard Gas Given 5 Days Before Injection of Sheep Blood		Mustard Gas Given at Same Time as Injection of Sheep Blood		Mustard Gas Given 5 Days After Injection of Sheep Blood		Control	
	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin
3	384	0	768	0	384	0	48	0
5	3,072	0	12,288	0	3,072	0	1,536	0
7	96	0	12,288	50	12,288	800	12,288	400
9	384	0	6,144	800	12,288	800	12,288	1,600
11	768	0	6,144	1,600	12,288	6,400	12,288	6,400
13	12,288	0	12,288	1,600	3,072	6,400	6,144	6,400
15	6,144	800	12,288	3,200	3,072	6,400	6,144	6,400
16	6,144	1,600	6,144	3,200	3,072	1,600	6,144	3,200
19	12,288	1,600	6,144	800	3,072	1,600	3,072	1.600
21	12,288	0	3,072	200	1,036	1,600	3,072	1,600
24	6,144	0	3,072	0	1,536	800	1,536	800

EXPER. 3.—The study was made on dogs and the antigen was sheep blood, 30 c c being injected intraperitoneally. The amount of mustard gas given was 0.5 c c 0.1% solution per kilo of weight. The titer of lysin only was determined, the serum being heated to 56 C. for 30 minutes and complemented in each case with 0.0125 c c of fresh guinea-pig serum, each mixture containing 2 c c of 5% sheep blood (washed), the requisite amount of heated dog serum, complement and salt solution to make 6 c c. The dogs that received mustard

gas 7 and 3 days before the antigen and those receiving it simultaneously with the antigen produced uniformly less lysin than the controls and the dogs that received mustard gas 3 and 7 days after the antigen. It is not possible to say whether in the last case the production of lysin was affected much or not. In all cases the fairly typical curves were described.

TABLE 3

EFFECT OF MUSTARD GAS ON LYSIN PRODUCTION IN DOGS INJECTED INTRAPERITONEALLY WITH 30 C C OF SHEEP BLOOD

Days After Injection of Sheep Blood	Mustard Gas Given 3 Days Before Injection of Sheep Blood	Mustard Gas Given at Same Time as Injection of Sheep Blood	Mustard Gas Given 3 Days After Injection of Sheep Blood	Controls
2	48	48	48	24
4	48	192	384	96
6	192	768	768	384
8	1,536	1,536	6,144	384
10	.....	12,288	6,144	768
12	3,072	12,288	6,144	3,072
14	3,072	3,072	3,072	12,288
16	3,072	1,536	1,536	12,288
18	1,536	1,536	1,536	6,144
20	1,536	1,526	1,536	3,072
22	1,536	1,536	1,536	3,072
24	1,536	1,536	1,536	3,072
26	768	1,536	1,536	1,536
28	768	Died	1,536	768
30	768		1,536	768
32	768		768	384
34	384		768	192
36	384		384	96
38	192		384	192

TABLE 4

EFFECT OF MUSTARD GAS ON AGGLUTININ PRODUCTION IN DOGS INJECTED INTRAVENOUSLY WITH 1 C C OF A 10% SUSPENSION OF RAT BLOOD PER KILO

Days After Injection of Rat Blood	Mustard Gas Given 7 Days Before Injection of Rat Blood	Mustard Gas Given at Same Time as Injection of Rat Blood	Mustard Gas Given 4 Days After Injection of Rat Blood	Controls
1	58	48	96	48
3	192	48	192	192
5	192	384	384	768
7	96	384	384	768
9	192	768	768	1,536
11	94	768	768	1,536
13	48	384	384	1,536
15	96	192	384	768
17	48	192	192	768
19	48	96	192	384
21	48	96	96	384
23	48	192	96	384
27	48	192	96	192
31		192	96	192
36		192	96	192

EXPER. 4.—In this experiment dogs were injected intravenously with 1 c c of a 10% suspension of rat blood per kilo of weight and the titer of the resulting agglutinin for rat corpuscles was determined. As before, mustard gas (0.5 c c, 0.1% per kilo) was given intravenously before and after and also simultaneously with the antigen injection. The results were definite. In the dogs given mustard gas before the antigen there was hardly any new

production of agglutinin; in the dogs injected with mustard gas and rat corpuscles at the same time the agglutinin production was much less than in the controls; and in the dogs injected with mustard gas after the injection of the antigen there was little, if any, interference with agglutinin accumulation in the blood as compared with the controls (table 4).

TABLE 5

LEUKOCYTES IN DOGS GIVEN LETHAL AND NONLETHAL INTRAVENOUS INJECTIONS OF MUSTARD GAS IN 50% GLYCEROL

Amount of Mustard Gas Given	Dog	Time of Blood Examination in Relation to Mustard Gas Injection	Leukocytes on the Basis of 100 Cells Counted									
			Total per C.Mm.	Polymorphonuclears (Arneth Scale)					Eosinophils	Basophils	Mononuclears	
				I	II	III	IV	V			Large	Small
0.25 c c 0.2% in 50% glycerol per kilo	1 12 kilos in wt.	Before	6,600	1	22	30	13	7	0	0	8	19
		6 hours	10,200	1	30	40	10	2	0	0	7	10
		1 day	8,200	0	28	23	20	7	0	0	8	14
		2 days	7,400	0	3	30	26	16	0	0	19	6
		3 days	4,600	1	4	23	32	6	0	1	10	23
		4 days	4,800	1	4	13	25	21	2	1	14	19
		5 days	3,200	2	3	13	23	25	1	1	8	24
		6 days	4,600	0	7	11	22	19	1	0	10	30
		7 days	6,400	1	15	24	19	21	1	0	4	19
		8 days	8,500	8	16	23	14	10	0	0	9	20
	3 20 kilos in wt.	Before	10,400	2	30	30	11	4	0	0	4	19
		6 hours	12,400	2	25	25	13	8	2	0	15	10
		1 day	9,400	0	25	21	22	1	1	0	17	13
		2 days	9,200	0	10	23	24	10	1	0	10	22
		3 days	8,600	0	14	28	18	7	2	0	23	8
		4 days	7,600	0	7	21	25	19	5	0	15	8
		5 days	6,400	1	6	21	16	23	2	0	10	21
		6 days	7,800	1	2	18	15	26	4	0	12	22
		7 days	8,800	0	16	24	21	19	3	1	16	18
		8 days	9,800	4	19	20	14	22	2	0	14	5
	5 14 kilos died 3d day	Before	8,200	4	30	26	21	10	0	1	5	3
		6 hours	12,600	0	35	35	8	1	0	0	8	13
		1 day	16,000	0	32	40	14	4	0	0	6	4
		2 days	4,800	1	24	36	17	6	0	0	8	8
		3 days	1,800	0	0	6	9	8	0	0	42	35
2 c c 0.2% in 50% glycerol per kilo	2 11 kilos died at en d of 2d day	Before	11,000	2	15	29	15	13	0	0	8	18
		6 hours	22,000	2	30	30	16	7	0	0	10	5
		1 day	26,800	0	30	35	18	8	0	1	4	4
		2 days	14,200	2	16	20	23	23	0	1	4	11
	4 15 kilos died 3d day	Before	6,800	3	20	23	20	6	1	0	7	20
		6 hours	10,800	4	30	30	16	8	0	0	7	5
		1 day	8,600	3	23	37	22	7	0	0	4	5
		2 days	5,400	0	5	26	25	27	0	0	7	10
		3 days	4,200	0	15	19	20	22	0	0	9	15
	6 11 kilos died 2d day	Before	15,400	1	20	31	24	17	0	0	5	2
		6 hours	25,000	0	42	33	11	5	0	0	4	5
		1 day	31,600	1	24	32	20	6	0	0	11	6
		1.3 day	25,400	2	26	32	21	11	0	0	2	6

As no results of leukocyte counts in dogs poisoned with mustard gas seem available, we insert here a table (5) giving the main results of our counts in connection with these experiments.

It is to be noted that mustard gas produces at first a decided increase in the circulating leukocytes (as high as 31,600 in a dog that died the next day); if death does not occur early the leukocytes begin to drop until a figure as low as 1,800 in dog 5 is reached just before death. In animals given a nonlethal dose, the leukocytes may drop and gradually increase again to about normal in about 8 days after injection.

The Arneth classification shows a moderate shift to the left during leukocytosis, which is followed by a shift to the right with the onset of leukopenia, reaching a point of almost total absence of leukocytes 1 and 2 as in dog 5, on the third day, shortly before death.

The mononuclear cells showed no significant change, except that in extreme cases of leukopenia there was marked relative lymphocytosis.

SUMMARY AND CONCLUSIONS

Mustard gas (dichlorethylsulphid) administered intravenously in amounts ranging from 0.005 to 0.0001 c c a few days before or coincident with the intraperitoneal injection of sheep blood to rabbits (ranging from 3 to 4 kilos in weight) had a restraining effect on the production of specific lysin and precipitin in these animals as compared to controls given sheep blood alone. The lysin curve ran about the same course as that in normal rabbits, but the latent period of the precipitin curve was much longer than normal. Given several days after the introduction of the antigen, there was still an appreciable effect of the mustard gas though less marked.

The lysin produced by dogs following the intraperitoneal injection of sheep's blood was also less after mustard gas had been given intravenously a week before and coincidently than when given a week after the antigen administration. The agglutinin for rat blood corpuscles formed by dogs after the intravenous injection of rat blood was also lessened by the intravenous injection of mustard gas (0.5 c c, 0.1% per kilo) before or coincident with the injection of the antigen. Little, if any interference, with agglutinin production was noted if the mustard gas was given after the rat blood.

Mustard gas profoundly modifies the leukocyte count of the blood in dogs as well as in rabbits and human beings (table 5).

These experiments tend to place mustard gas in a class with the leukotoxic agents: benzene, the radioactive preparations of which thorium X is an example, and the roentgen ray, all of which, though probably differing in the details of their mechanism, have a profound effect on the hematopoietic organs, the leukocytes and specific antibody formation.